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Ofloxacin and Penicillin G Combination Therapy in Prevention of Bacterial Translocation and Animal Mortality after Irradiation

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The efficacies of 40 mg of ofloxacin per kg/day given orally and 250 mg of penicillin per kg/day given intramuscularly, alone or in combination, were evaluated in the prevention of mortality of C3H/HeN female mice given 8.2 Gy of ^{60}Co radiation. Mortalities were 51 of 60 mice (85%) in the control group, 46 of 60 mice (77%) among those treated with penicillin, 32 of 60 mice (53%) among those treated with ofloxacin ($P < 0.05$), and 5 of 60 mice (8%) among those treated with ofloxacin and penicillin ($P < 0.001$). The organisms recovered from the livers of control mice were members of the family *Enterobacteriaceae* and *Streptococcus* spp. A reduction in the number of the *Enterobacteriaceae* was noted only in ofloxacin-treated mice, and a reduction in the number of *Streptococcus* spp. was noted only in the penicillin-treated mice. Reductions in the numbers of both groups of organisms were noted only in the animals treated with both agents. This study shows the advantage of the combination of ofloxacin and penicillin in the prevention of bacterial translocation and animal mortality after irradiation.

Ionizing radiation enhances susceptibility to systemic bacterial infections caused by endogenous and exogenous organisms (4, 6). One source of endogenous infections is the bacterial gastrointestinal tract flora (4). Following irradiation, members of that flora translocate to the liver and spleen and can be associated with fatal septicemia (4, 5). The most important bacterial species isolated from septic animals are members of the family *Enterobacteriaceae* and *Streptococcus* spp. (4, 5). Prevention of bacterial translocation in irradiated animals and control of the subsequent sepsis by these organisms enhance survival in models of experimental infection (3).

In previous studies, we found the quinolone antibiotics to be efficacious in controlling systemic infections following irradiation (2, 3). The efficacies of these agents are believed to be due to their selective ability to eradicate members of the family *Enterobacteriaceae* while preserving the anaerobic gut flora. However, animal mortality was not completely prevented in those studies, and quinolone-resistant *Streptococcus* spp. were isolated in the organs of animals that succumbed to infection.

This study was designed to evaluate the efficacy of supplementing the antimicrobial therapy of irradiated mice with a quinolone with penicillin, which is effective against *Streptococcus* spp. We found that combined antibiotic therapy with ofloxacin and penicillin prevented bacterial translocation to the liver and resulted in 90% survival of mice given lethal doses of radiation.

Female C3H/HeN mice (age, approximately 12 weeks) were obtained from the National Cancer Institute Animal Breeding Facility (Frederick, Md.). Animals were maintained as described previously (3). The mice were provided commercial rodent chow and acidified water (pH 2.2) that was changed to tap water 48 h before irradiation. All experimental procedures were done in compliance with National Institutes of Health and Armed Forces Radiobiology Research Institute guidelines regarding animal use and care.

Mice were placed in Plexiglas restrainers and given a whole-body dose of 8.2 Gy of radiation at 0.4 Gy/min from a ^{60}Co source. The dose rate was determined at the midline, as described previously (3). The tissue-air ratio in this experiment was 0.98.

The lethal dose for 50% of C3H/HeN female mice 30 days after exposure was 7.9 Gy. The dose of 8.2 Gy is a lethal dose, and it was used because survival (80 to 90% in 30 days) from radiation-induced hematopoietic damage is possible if antibacterial treatments are successful.

The antibiotics used were ofloxacin (Ortho Pharmaceutical Corp., Raritan, N.J.) and procaine penicillin G (Wyeth Laboratories, Philadelphia, Pa.). Both antibacterial agents were given once every 24 h. Ofloxacin was given by oral gavage in a dose of 40 mg/kg/day in a volume of 0.1 ml of distilled water. Procaine penicillin was administered by intramuscular (i.m.) injection to alternate thighs in a dose of 250 mg/kg/day in a volume of 0.1 ml of saline. All control animals received 0.1 ml of sterile distilled water by oral gavage and 0.1 ml of normal saline i.m.

Concentrations of the antibiotics in serum were determined in each of six irradiated mice 1 and 23.5 h after the administration of the antimicrobial agents on day 5 of therapy. *Bacillus subtilis* ATCC 6633 was used as a test organism in a Mueller-Hinton agar (pH 7.4).

Mice were observed for mortality and symptoms of disease for 30 days. Five mice were selected at random from each group on days 4, 6, 8, 10, and 12 following irradiation. When fewer than five mice in a group survived, all mice were studied that day. Mice were euthanized by cervical dislocation. Specimens of livers were processed for the presence of bacteria. No other organs were examined and no blood samples were obtained, because studies showed that positive liver cultures correlate best with sepsis (4). The livers were aseptically removed and homogenized immediately. The liver specimens were swabbed onto blood and MacConkey agars, and the organisms were identified by conventional

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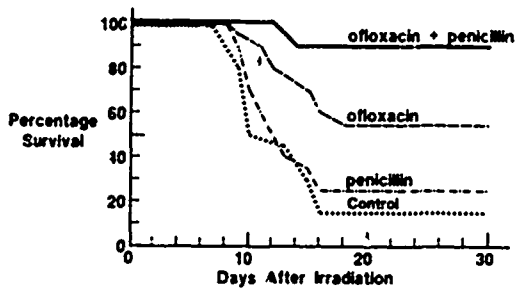

FIG. 1. Survival of 80 C3H/HeN mice irradiated with 8.2 Gy of ^{60}Co and treated orally with ofloxacin and i.m. with procaine penicillin G. Twenty mice were included in each group. The data represent results of one experiment; an identical experiment showed similar data (see text).

methods (9). The susceptibilities of the isolates were determined by the Kirby-Bauer method.

Antimicrobial therapy was initiated 72 h after irradiation, and antimicrobial agents were administered for 10 days. A total of 200 mice were included in each of the three replicate experiments comprising both survival (80 mice) and bacterial translocation (120 mice) studies. However, microbial analysis of the liver was done only twice. Each experiment consisted of three antibiotic therapy groups and the water-saline-treated control group. Each group consisted of 50 mice: 20 were observed for mortality and 30 were used for cultures of liver on each of 5 designated days. The first group of mice received ofloxacin, the second group received penicillin, the third group received a combination of ofloxacin and penicillin, and the fourth group received distilled water orally and saline i.m.

Statistical analyses were done by the Cox-Mantel test (8). Mortalities in the groups that received ofloxacin or ofloxacin and penicillin were significantly ($P < 0.05$) less than those in the saline- or penicillin-treated groups (Fig. 1). The mortalities in all experiments were similar, and the data were therefore pooled. Of the 60 water-saline-treated mice, 51 (85%) died, 46 of the 60 (77%) penicillin-treated mice died, 32 of 60 (53%) ofloxacin-treated mice ($P < 0.05$) died, and 5 of 60 (8%) mice treated with ofloxacin and penicillin ($P < 0.001$) died.

Most of the organisms were isolated on days 8, 10, and 12 after irradiation (Table 1). Analysis of the data obtained on those days showed recoveries of members of the family *Enterobacteriaceae* in 11 of 13 (85%) of the livers of control mice and *Streptococcus* spp. in 6 of 13 (46%) of the livers of control mice. No other types of isolates were recovered. No significant reduction in the rate of isolation was noted in the recovery of the *Enterobacteriaceae* in penicillin-treated mice (7 of 12 mice). However, the number of livers that harbored *Streptococcus* spp. was reduced to 1 of 12 (8%; $P < 0.05$). The recovery rate of the *Enterobacteriaceae* was reduced in the ofloxacin-treated mice (1 of 14 mice; $P < 0.05$); however, the rate of isolation of *Streptococcus* spp. was not altered (8 of 14; 57%). Therapy with ofloxacin and penicillin reduced the rate of recovery of both the *Enterobacteriaceae* (1 of 15 mice) and *Streptococcus* spp. (0 of 15 mice).

In the second experiment, the *Enterobacteriaceae* were isolated in 12 of 14 (86%) of the livers of control mice, and *Streptococcus* spp. were recovered in 7 of 14 (50%) of the livers of control mice. As in the first experiment, no reduction was noted in the isolation rate of the *Enterobacteriaceae*

TABLE 1. Recovery of bacteria from livers of C3H/HeN mice given 8.2 Gy of ^{60}Co and treated with ofloxacin and penicillin

Organism and treatment	No. of animals with bacteria/no. of animals studied on the following day after inoculation[a]:				
	4	6	8	10	12
Enterobacteriaceae family					
Control	0/5	1/5	3/5	5/5	3/3
Penicillin	0/5	1/5	2/5	3/5	2/2
Ofloxacin	0/5	0/5	0/5	0/5	1/4
Ofloxacin and penicillin	0/5	0/5	0/5	0/5	1/5
Streptococcus spp.					
Control	0/5	1/5	2/5	3/5	1/3
Penicillin	0/5	0/5	0/5	1/5	0/2
Ofloxacin	0/5	0/5	3/5	2/5	3/4
Ofloxacin and penicillin	0/5	0/5	0/5	0/5	0/5

[a] The data represent results of one experiment; one replicate showed similar data (see text).

in penicillin-treated mice (6 of 11 mice; 55%), but the number of livers harboring *Streptococcus* spp. was reduced to 1 of 11 (9%; $P < 0.05$). The recovery rate of the *Enterobacteriaceae* was reduced in ofloxacin-treated mice (1 of 13 mice; $P < 0.05$); however, the rate of recovery of *Streptococcus* spp. was unchanged (7 of 13; 54%) A significant reduction was noted in the recovery of both the *Enterobacteriaceae* (0 of 15 mice) and *Streptococcus* spp. (2 of 15 mice; 13%) in mice treated with ofloxacin and penicillin ($P < 0.05$). All the *Enterobacteriaceae* were susceptible to ofloxacin and resistant to penicillin. All *Streptococcus* spp. were susceptible to penicillin and resistant to ofloxacin.

The mean concentrations of ofloxacin in serum were 2.4 ± 0.3 μg/ml at 1 h and 0.4 ± 0.2 μg/ml at 23.5 h. The mean concentrations of penicillin G were 38.5 ± 4.6 μg/ml at 1 h and 6.2 ± 2.5 μg/ml at 23.5 h.

This is the first study demonstrating that, although the quinolone ofloxacin can reduce mortality following exposure to radiation, the addition of penicillin can further reduce mortality. Although ofloxacin decreased the translocation of the *Enterobacteriaceae*, *Streptococcus* spp. continued to disseminate to the liver, and mortality was not prevented in almost half of the animals. While penicillin was ineffective in controlling mortality by itself, its addition to ofloxacin was successful in controlling the spread of *Streptococcus* spp. and reduced mortality even further.

Several quinolones have been used empirically in immunocompromised patients to decrease the colonization of the gastrointestinal tract and the systemic spread of the *Enterobacteriaceae* (10). The efficacies of these agents are due to their activities against the *Enterobacteriaceae* and their lack of activity against anaerobic bacteria (1). Furthermore, since the quinolones are also absorbed from the gastrointestinal tract, they can eradicate any susceptible pathogens that have spread systemically. Although prophylactic quinolone therapy reduced the occurrence of bacteremia caused by the *Enterobacteriaceae* in immunocompromised patients, it did not prevent infection with *Streptococcus* spp. or reduce the subsequent mortality from infection (7).

The findings of this study suggest that the addition of penicillin therapy directed at the organisms that are not inhibited by quinolone therapy may prevent their translocation and the subsequent development of infection with these quinolone-resistant organisms. These findings support the

preliminary observations in neutropenic patients, in whom the addition of penicillin to a quinolone prevented treatment failures with the quinolone caused by streptococci (7). Another possible explanation for the synergistic effect of adding penicillin to ofloxacin is that penicillin acts synergistically against the *Enterobacteriaceae*, which are the major pathogens. Further studies of patients to explore the usefulness of this approach in controlling bacterial infection in the immunocompromised host are indicated.

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